

Mumunine - A New Carbazole Alkaloid from *Murraya koenigii* (Linn.) Spreng

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Abstract

The plant *Murraya koenigii*, commonly known as curry leaf tree is a rich source of carbazole alkaloids. A number of monomeric as well as dimeric carbazoles with C₁₃, C₁₈ and C₂₃ skeleton have been isolated from the plant. In my present work, a new carbazole alkaloid, designated as mumunine, was isolated from the bark of *Murraya koenigii* (Linn) Spreng, along with a known carbazole alkaloid, viz. mahanimbine. The structure of the new alkaloid **1** was elucidated on the basis of 1D and 2D NMR spectral data analysis. In this paper, the isolation and structure elucidation of the new compound will be discussed in detail.

Keywords: *Murraya koenigii*; Rutaceae; Carbazole alkaloids; Mumunine; 2D NMR.

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1. Introduction

Many of the medicinally important plant-derived pharmaceuticals have been essential in the era of modern medicine and some of these substances, such as morphine have attained the official status of strategic materials. However, despite these many important past contributions from the plant kingdom, a great number of many plant species have never been described and remain unknown to science and relatively few have been surveyed systematically to any extent for biologically active chemical constituents. Thus, it is reasonable to expect that new plant sources of pharmaceutically interesting materials remain to be discovered and developed.

India and neighbouring countries like Sri Lanka, China, Bangladesh etc. are very rich sources of medicinal plants. Many researchers from these countries are working hard in search of bioactive organic substances from their natural resources. Penu *et al.* have recently published a paper on investigation of the phytochemicals and their antioxidant, antimicrobial and thrombolytic activities and also estimated total phenolic and flavonoid contents of *Pandanus odoratissimus* (*P. odoratissimus*) leaves of methanol extract [1].

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Garuba *et al.* have published a paper on *Ganoder malucidum*, which is a mushroom commonly used in folk medicine especially Traditional Chinese Medicine (TCM) but information on its nutritional and chemical profiles were insufficient [2]. Nowadays, more and more researches on natural products are in high demand in the global market.

The plant *Murraya koenigii* (L.) Spreng (Fig. 1) belonging to the family Rutaceae is native to India and now distributed in most of southern Asia. The leaves of this plant are well-known as curry leaves and have been used as one of the important herbs of south Indian cooking. Various parts of the plant have been used in traditional medicine for the treatment of headache, toothache and stomachaches, influenza, rheumatism, traumatic injury, and insect and snake bites, and as an antidiarrhoeic as well as an astringent. Intake of the leaves can increase digestive secretions and relieve nausea, indigestion and vomiting [3]. The leaves and bark are used in analgesia and local anesthesia and for the treatment of eczema and dropsy [4]. Chloroform extract of the root bark of *M. koenigii* displayed significant cytotoxic activity against cultured keratin B (KB) cell.



Fig. 1. Image of the plant *Murraya koenigii* Spreng

Murrayanine is the first carbazole alkaloid isolated from the stem bark of *M. koenigii*. After that a number of carbazole alkaloids have been isolated from this plant, possessing C_{13} , C_{18} and C_{23} skeletons [5-8]. A number of derivatives of these carbazole alkaloids were also prepared, many of which showed potent biological activities [9-11].

During the present investigation on the leaves and bark of *M. koenigii*, two carbazole alkaloids were isolated from the bark of the plant, a novel isolate **1** (Fig. 2) was designated as mumunine. During structure elucidation of the isolated compounds by ^1H and ^{13}C NMR techniques, the chemical shifts of all the compounds were assigned unambiguously. This

presents the isolation and structure elucidation of the novel carbazole alkaloid **1**. Compound **2** (Fig. 2) was found to be mahanimbine by comparison of its spectral data with literature [12].

2. Materials and Methods

2.1. Drugs and chemicals

Different solvents such as methanol, petroleum ether, chloroform and other necessary chemicals and reagents were of highest analytical grade and collected from Annapurna Chemicals and Equipments, Kolkata, India.

2.2. General experimental procedure

TLC was carried out on silica gel 60 F₂₅₄ (Merck, Germany) plates and spots were visualized by adsorption of iodine. Column chromatography was performed on silica gel mesh 60-120 (Merck). The mass spectra were recorded on a Q-TOF-Micromass spectrometer. ¹H NMR and ¹³C NMR spectra were recorded using a BRUKER AVANCE 600 MHz NMR with TLC-cryoprobe using TMS as internal standard. Data are presented as follows: Chemical shift (in ppm on the δ scale relative to $\delta_{\text{TMS}} = 0$), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br. = broad), coupling constant (J/Hz). ¹H NMR and ¹³C NMR spectra were recorded at both 300 MHz and 600 MHz.

2.3. Plant material

The dried leaves and stem bark of *Murraya koenigii* were collected from Taki, North 24 parganas, West Bengal (India). A voucher specimen has been deposited at Taki Govt. College, Taki, North 24 parganas.

2.4. Extraction and isolation

1 kg of powdered and dried stem bark of *Murraya koenigii* was extracted with methanol at room temperature and it was fractionated into three parts - petroleum ether, chloroform and ethyl acetate. The chloroform fraction (10 g) was chromatographed on a column of silica gel (mesh size 60-120). Gradient elution was carried out with petroleum ether followed by various mixtures of petroleum ether-benzene (3:1, 1:1 and 1:3) and benzene-chloroform (3:1, 1:1 and 1:3), 200 mL each. Compound **1** was eluted from this column with a 1:1 mixture of petroleum ether-benzene. TLC with benzene as mobile phase showed it to be a pure compound.

Fraction no. 5 (Pet. ether:benzene = 1:1) eluted from the first column was re-chromatographed on a column of silica gel (mesh size 60-120). Gradient elution was carried out with petroleum ether followed by various mixtures of petroleum ether-benzene

(3:1, 1:1 and 1:3), 200 mL each. Compound **2** was eluted from this column with a 3:1 mixture of petroleum ether-benzene. TLC with benzene as mobile phase showed it to be a pure compound.

2.5. Characterisation of *mumunine* (**1**)

Off-white amorphous solid, molecular formula $C_{20}H_{21}O_2N$. Positive ion TOFMS m/z 316 [(M-Me)+Na⁺+H⁺](100%). ¹³C and ¹H NMR spectral data: Table 1.

2.6. Characterisation of *Mahanimbine* (**2**)

White amorphous solid, molecular formula $C_{23}H_{25}NO$. Positive ion TOFMS m/z 354[(M+Na⁺)(100 %). ¹³C and ¹H NMR spectral data: Table 2.

3. Results and Discussion

The CHCl₃ fraction of methanol extract of the stem bark of *M. Koenigii* on chromatographic resolution over silica gel (60-120 mesh) yielded **1** and **2**, the structures of **1-2** (Fig. 2) were elucidated on the basis of 1D and 2D NMR (¹H-¹H COSY, HSQC, HMBC and NOESY) spectral data analysis (Table 1).

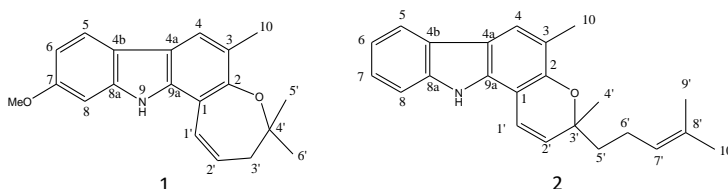


Fig. 2. Structure of the isolated compounds.

Mumunine **1** has a molecular formula $C_{20}H_{21}O_2N$ (M^+ :307) derived from m/z 316[(M-Me)+Na⁺+H⁺] indicated in the Q-TOF micromass spectroscopy. The ¹³C NMR spectrum of **1** (Fig. 6, Table 1) displayed signals for 20 carbons. The ¹H NMR spectrum (Fig. 5, Table 1) exhibited signals for four aromatic methine protons, of which one appeared as singlet, two as doublets and one as multiplet. Their exact positions were confirmed by ¹H-¹H COSY (Fig. 4). The ¹H NMR spectrum also showed singlet for one aromatic methyl (δ 2.36), one methoxy group (δ 3.90) and one gem-dimethyl group (δ 1.48).

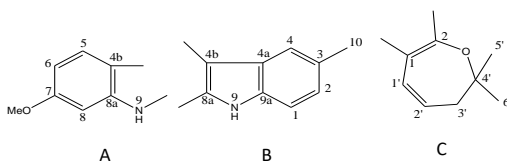
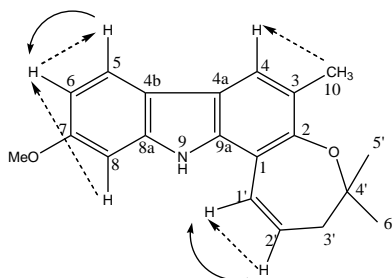
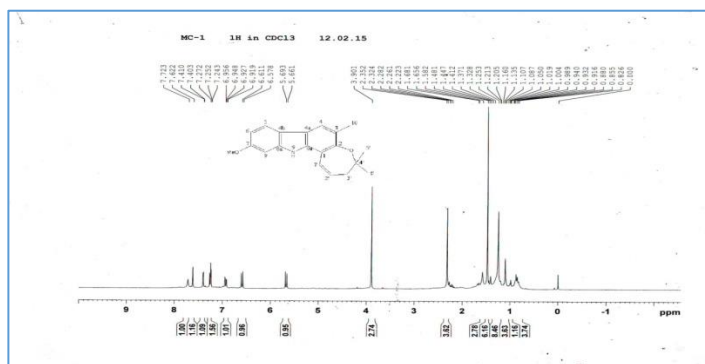


Fig. 3. Part structures of compound (**1**) derived from HMBC data and ¹³C NMR data.

Fig. 4. Some important COSY (bold lines) and NOESY (dotted lines) of compound **1**.Table 1. One-bond (^1H - ^{13}C HSQC) and multiple bond (HMBC) correlation data of compound **1**.

Carbon	^{13}C ppm	NMR(δ in ppm)	^1H NMR(HSQC)	HMBC
C-1	104.5(s)	-	-	117.2, 129.3
C-2	149.8(s)	-	-	121.2, 117.2
C-3	118.4(s)	-	-	16.1
C-4	121.2(d)	-	δ 7.62 (1H,s)	124.4, 135.7, 149.8, 16.1
C-4a	116.9(s)	-	-	102.6
C-4b	124.4(s)	-	-	121.2, 111.0
C-5	102.6(d)	-	δ 7.41 (1H,d,J=2.1)	113.0, 134.3, 153.9
C-6	111.0(d)	-	δ 7.26 (1H,t,J=6)	153.9, 124.4
C-7	153.9(s)	-	-	102.6, 111.0, 113.0, 56.0
C-8	113.0(d)	-	δ 6.94 (1H,dd,J=2.4)	102.6, 134.3, 153.9
C-8a	134.3(s)	-	-	102.6, 113.0
C-9a	135.7(s)	-	-	121.2, 117.2
C-10	16.1(q)	-	δ 2.33 (3H,s)	149.8, 121.2, 118.4
C-1'	117.2(d)	-	δ 6.60 (1H,d,J=9.6)	135.7, 149.8, 104.5, 75.8, 27.6
C-2'	129.3(d)	-	δ 5.68 (1H,d,J=9.6)	104.5, 75.8, 27.6
C-3'	29.7(t)	-	δ 1.25 (2H,s)	-
C-4'	75.8(s)	-	-	117.2, 129.3, 27.6
C-5'	27.6(q)	-	δ 1.48 (3H,s)	129.3
C-6'	27.6(q)	-	δ 1.48 (3H,s)	129.3
CH ₃ O-7	56.0(q)	-	δ 3.90 (3H,s)	153.9

Fig. 5. ^1H -NMR spectra of compound **1**.

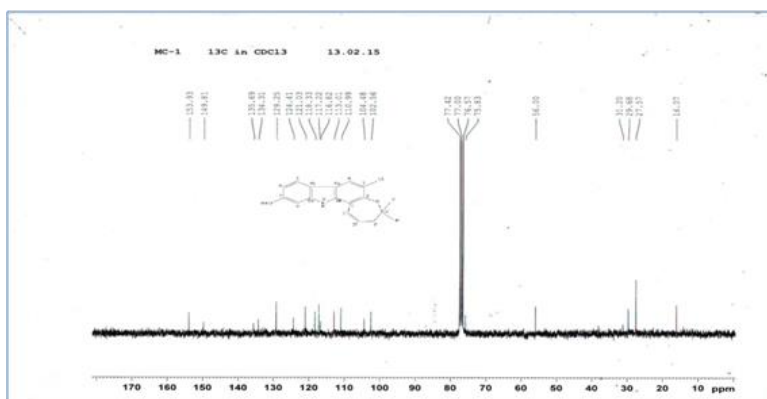


Fig. 6. ^{13}C -NMR Spectra of compound **1**.

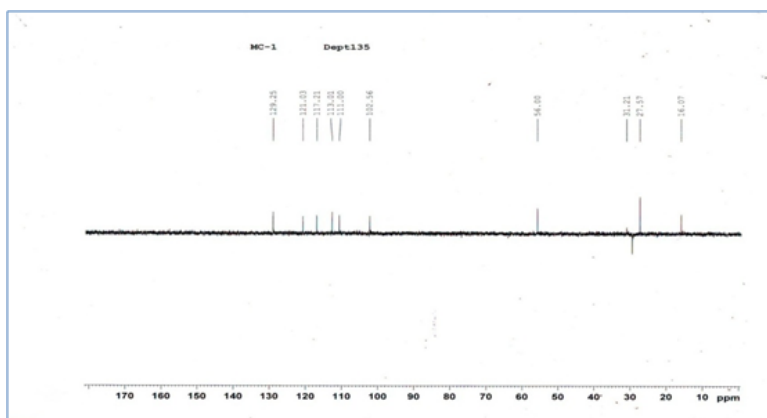


Fig. 7. DEPT 135 spectra of compound **1**.

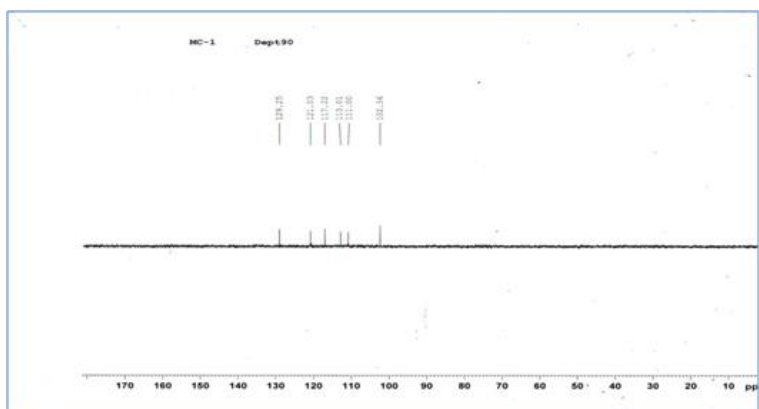


Fig. 8. DEPT 90 spectra of compound **1**.

Mahanimbine, **2** (C₂₃H₂₅NO), is the first member of the group of carbazole alkaloids with a C₂₃- skeleton. The ¹H NMR spectrum (Table 2) of **2** showed the presence of five aromatic protons at δ 7.66, 7.87, 7.36, 7.28 and 7.17. δ 2.33 was assignable to one aromatic methyl group. The signal for the three proton singlet at δ 1.45 (4'-CH₃) together with the symmetrical doublets at δ 5.66 (H-1') and 6.65 (H-2') (J = 9.6 Hz each) suggested the presence of a 2,2-dimethyl-Δ³-pyran skeleton and the C₅H₉ residue containing an unsaturation was confirmed by the presence of two sets of methylene protons at δ 1.77 (5'-CH₂) and 2.16 (6'-CH₂), the multiplet at δ 5.11 (H-7') and the gem-dimethyl groups at δ 1.66 and 1.58.

Compound **2** displayed 23 signals in the ¹³C NMR spectrum (five aromatic doublets, one aromatic and one aliphatic methyl, one gem-dimethyl, one oxygen-bearing quaternary carbon, seven aromatic singlets, three methines in the pyran ring and side chain, two methylene groups and one aliphatic quaternary carbon). The structure of **2** was finally established as mahanimbine by the comparison of the reported spectral data (¹H NMR of the compound isolated from the same plant by earlier workers) [12].

Table 2. ¹H and ¹³C NMR spectral data of compound **2**.

Carbon	¹³ C NMR(δ in ppm)	¹ H NMR(δ values)
C-1	104.2	-
C-2	149.9	-
C-3	117.0	-
C-4	121.2	7.66,s
C-4a	118.0	-
C-4b	123.9	-
C-5	119.3	7.87,d
C-6	110.3	7.36,m
C-7	124.1	7.17,m
C-8	119.5	7.28,d
C-8a	139.4	-
C-9a	134.8	-
C-10	16.1	2.33,s
C-1'	117.5	6.65,d
C-2'	128.5	5.66,d
C-3'	78.1	-
C-4'	25.8	1.45,s
C-5'	40.7	1.77,m
C-6'	22.7	2.17,m
C-7'	124.2	5.11,m
C-8'	131.7	-
C-9'	17.6	1.66,m
C-10'	25.7	1.58,m

4. Conclusion

The plant *Murraya koenigii* is a well-known medicinal plant that is found in various parts of India, as well as in Asia. The plant is a rich source of carbazole alkaloids and many of them showed potent biological activities. This paper is on the isolation and structure

elucidation of a new compound isolated from this plant. This will definitely enrich the literature on carbazole alkaloids and in future, this alkaloid can also be tested for biological activities. Researchers can also prepare different derivatives of the new alkaloid and those can also be tested in search of bioactive molecules.

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